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Title: CORK AS A SUSTAINABLE CARBON SOURCE FOR NATURE-BASED SOLUTIONS TREATING HYDROPONIC WASTEWATERS - PRELIMINARY BATCH STUDIES

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Abstract: Reusing by-products is an important strategy regarding preservation of natural capital and climate change mitigation. Therefore, this study aimed to evaluate the potential cork granulated, a by-product of winery industry, as an organic carbon source for the treatment of hydroponic wastewater. First, chemical characterization was performed and discussed. Second batch studies were performed using synthetic hydroponic wastewater to understand the role of particle size (PS), pH and contact time (CT) on the release of organic carbon. The suberin is the major compound, representing more than 50%. It was noticed that might be a variance on the content of suberin across species, within the same species and depending on the extraction part (Belly, cork and back). More than 60% of the sample is composed by carbon while less than 1% was nitrogen, (high C:N ratio), indicating a low risk of releasing organic nitrogen. The statistical results suggested that the main effect of PS on the release of organic carbon is greater than both, CT and pH. The chemical release of organic carbon gets slower with time, being this effect greater as the PS increase. Moreover, estimations showed that by using the PS 4mm the amount of water treated would be twice the amount if the PS 8 mm had been used. The PS, seem to play an important role at design nature-based solution focused on denitrification. The surface response methodology indicates a significant negative interaction between CT and PS suggesting that the mathematical model could be used for further optimization studies. The reuse of organic by-products as filter medias seems to be an environmental and economic friendly alternative to enhance denitrification in nature-based solutions while preserving natural capital. However, further real scale and long-term experiments are needed to validate cork`s potential as an "internal" organic carbon source for nature based solutions.

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Barcelona, June 13th, 2018

Dear Editor Paola Verlicchi

I am pleased to submit an original research article entitled "Cork as a sustainable carbon source for nature-based solutions treating hydroponic wastewaters – preliminary batch studies" for consideration for publication in the journal ***Science of total Environment - VSI: WETPOL2017***.

We believe that this manuscript is appropriate for publication mainly because highlights the importance of reusing by products in the scope of Nature-Based Solutions treating wastewaters. The reuse of organic by-products as filter medias seems to be an environmental and economic friendly alternative to enhance denitrification in Nature-Based Solutions while preserving natural capital. Moreover, the results suggest that particle size besides being an important parameter regarding the hydraulic design, also can play an important role at designing Nature-Based Solution focused on enhancing the removal of nitrogen by denitrification.

Considering the aims and scopes of the Journal, we believe that the paper connects the following spheres: Hydrosphere, Lithosphere and Biosphere. Mainly by proposing the reuse of cork, an organic by product as a carbon source (preserving Lithosphere) to enhance microbiological removal of nitrogen in nature based solution (Biosphere) treating hydroponic wastewaters (Hydrosphere).

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose. If you feel that the manuscript is appropriate for your journal, we suggest to consider experts from the fields of chemistry and constructed wetlands for peer reviewing.

Thank you for your consideration!

Sincerely,

Joana Castellar

Joana América Castellar da cunha
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***Graphical Abstract**

Hydroponics

Wastewater
High [NO_3^--N]
Low carbon

Integrating
production chains

Winery industry

By-product

Nature
Based
solutions

Further
research

New application
"Internal" carbon source

- Enhanced denitrification potential
- **Particle size** has a strong effect on **organic carbon release**

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- Reusing by products like cork as an organic carbon source for denitrification seems to be a sustainable alternative to enhance the efficiency of nature-based solutions treating hydroponic wastewaters.
- Organic carbon release slows along time, being the effect stronger as the particle size increases.
- Estimations showed a denitrification potential of 3.9 m^3 (cork 4 mm) and 1.8 m^3 (cork 8 mm) of hydroponic wastewater.
- The results have shown that particle size is an important parameter to design nature-based solutions when enhancing denitrification.

CORK AS A SUSTAINABLE CARBON SOURCE FOR NATURE-BASED SOLUTIONS TREATING HYDROPONIC WASTEWATERS – PRELIMINARY BATCH STUDIES

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Abstract

Reusing by-products is an important strategy regarding preservation of natural capital and climate change mitigation. Therefore, this study aimed to evaluate the potential cork granulated, a by-product of winery industry, as an organic carbon source for the treatment of hydroponic wastewater. First, chemical characterization was performed and discussed. Second batch studies were performed using synthetic hydroponic wastewater to understand the role of particle size (PS), pH and contact time (CT) on the release of organic carbon. The suberin is the major compound, representing more than 50%. It was noticed that might be a variance on the content of suberin across species, within the same species and depending on the extraction part (Belly, cork and back). More than 60% of the sample is composed by carbon while less than 1% was nitrogen, (high C:N ratio), indicating a low risk of releasing organic nitrogen. The statistical results suggested that the main effect of PS on the release of organic carbon is greater than both, CT and pH. The chemical release of organic carbon gets slower with time, being this effect greater as the PS increase. Moreover, estimations showed that by using the PS 4mm the amount of water treated would be twice the amount if the PS 8 mm had been used. The PS, seem to play an important role at design nature-based solutions focused on denitrification. The surface response methodology indicates a significant negative interaction between CT and PS suggesting that the mathematical model could be used for further optimization studies. The reuse of organic by-products as filter medias seems to be an environmental and economic friendly alternative to enhance denitrification in nature-based solutions while preserving natural capital. However, further real scale and long-term experiments are needed to validate cork`s potential as an "internal" organic carbon source for nature-based solutions.

Keywords
wastewater, denitrification, reusing, by-products, particle size, contact time.

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Abbreviations
Particle Size: PS
Contact Time: CT
Point of Zero Charge: PSZ
Fourier Transform Infrared Spectroscopy: FTIR
Constructed Wetlands: CWs
Nature-Based Solutions: NBS

1. INTRODUCTION

The use of urban and soilless agriculture is becoming more common in the past years to supply the ever-increasing food demand and to deal with water/land scarcity, leading to pollution potential. Wastewater from greenhouses, besides having high concentration of nitrates and phosphates are usually drained and discharged to the environment without proper treatment (Prystay and Lo, 2001). The leaching of N and P causes several environmental impacts such as, contamination of groundwater, eutrophication of surface waters and losses of ecosystem biodiversity (Oenema et al. 2011). Therefore, the treatment of wastewaters generated by soilless agriculture may play an important role, with regards of ensuring food security, sustainable management of water resources and environmental protection, once this type of agriculture can be implemented at both, urban and rural environments.

Conventional wastewater treatments such as, reverse osmosis, ion exchange, electrodialysis, and ultrafiltration are efficient, however have high maintenance and operation costs (Koide and Satta, 2004; Gagnon et al., 2010; Park et al., 2015). Therefore, nature-based solutions (NBS), such as constructed wetlands (CWs) and denitrification filters, may represent a sustainable and low-cost alternative to remove nitrogen from hydroponic wastewaters before discharge (Park et al., 2008; Gagnon et al., 2010; Abbassi et al.,2011; Park et al., 2015).

However, nitrogen removal from hydroponic wastewaters, by using NBS can be a challenge, since this water is known to have high concentration of nitrates and low carbon (Prystay and Lo, 2001). The availability of carbon is one of the main limiting factors regarding the efficiency of biological denitrification (Vymazal, 2007a; Wu et al., 2014; Mutsvangwa and Matope, 2017). According to Mutsvangwa and Matope (2017) and Amy et al. (2008) wastewaters with low carbon to nitrogen ratio, usually require an external carbon source to improve denitrification. However, the use of external carbon sources such as, methanol, ethanol, acetic acid and fructose besides increasing operational costs can cause negative environmental impacts (Park et al., 2008).

Therefore, alternative organic materials such as, plant biomass (Wen et al., 2010; Zhang et al., 2014), flower straws (Chang et al., 2016) and plant pruning (Park et al., 2008) have been proposed as an external carbon source, mainly because of their low cost, availability and renewable biomass. In addition, in the past 5 years, authors have shown the potential of roots exudates as a carbon source for denitrification (Zhai et al., 2013; Chen et al., 2016; Wu et al., 2017).

Moreover, some authors have suggested the use of organic filter media to enhance denitrification for NBS treating wastewaters, such as a pond culture with mud (Erbanová et al., 2012), bioreactor with woodchip (Nordström and Herbert, 2017), green wall with coconut fibber and light expanded clay (Masi et al., 2016) and green walls with Coco coir (Prodanovic et al., 2017). Results of Prodanovic et al. (2017) indicated that biological processes are enhanced by the addition of organic substrate. The coco coir increased the retention time, and thus, enhanced at the same time the microbiological removal processes. On the other hand, the increase of retention time can lead to an accumulation of total nitrogen in the effluent. The study of Masi et al., (2016) showed an increment of total Kjeldahl nitrogen when using coconut fibber as substrate and light expanded clay, possibly by the increment of retention time, which favours the release of organic compounds, such as organic nitrogen.

Nevertheless, reusing organic by-products, as filter media transforms what was once an external source, into an integrated part of the system, reducing operation costs while preserving natural capital. In this regard, cork granulated seems to have potential to be used as a sustainable “internal” organic source for the treatment of hydroponic wastewaters.

Cork by-product is generated from several operations of wine industry and it is considered as a natural, renewable, biodegradable raw material (Olivella et al., 2011a; Ramos et al., 2014; Boschmonart, 2011). The cork oak trees are planted, the bark is stripped for the first time when tree is 20 to 25 years old; the next stripping are carried out every 9 to 12 years (Boschmonart, 2011), with an expected productive life from 150 to 300 years depending on the tree’s health. In the Iberian Peninsula, the annual production of cork waste reaches 50.000 tons,

corresponding on average 40% of the cork processing industry that is discarded and sent to landfill.

Moreover, several researches have shown the potential of cork to remove contaminants such as, polycyclic aromatic hydrocarbons (Olivella et al., 2011a), methyl orange (Krika and Benlahbib, 2015), ofloxacin (Crespo-Alonso et al., 2013), Biphentrin (Domingues et al., 2005) ibuprofen, carbamazepine and clofibric acid (Dordio et al., 2011) or heavy metals (Pintor et al., 2012). On the other hand, not much is known about the behaviour of cork regarding the release of organic carbon.

The main goal of this paper was to investigate the potential of granulated cork as an organic carbon source. The chemical release of organic carbon can play an important role regarding the establishment of biofilm in natural wastewater treatments. Moreover, the chemical organic carbon released by the substrate can enhance denitrification process while reducing the use of external carbon sources and thus, ensuring a long-term performance on pollutant removal, reducing operation costs and environmental hazard. The granulated cork was characterized (PZC, FTIR, chemical and elemental constitution) and batch studies were performed using synthetic hydroponic wastewater in order to understand the role of PS, pH and CT on the release of organic carbon.

2. MATERIALS AND METHODS

2.1 Synthetic Hydroponic Wastewater

The composition of hydroponic wastewaters varies according to the crops, type of fertilizers used for the nutrition solution, frequency of application, time of the year and type of system (closed or open). A literature research was made to establish a reliable range of contaminants to guide the preparation of synthetic hydroponic water (Table 1). The compounds used to prepare the solution were potassium Nitrate (KNO_3), calcium chloride dihydrate ($CaCl_2*2H_2O$), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), sodium hydroxide (NaOH), magnesium sulphate heptahydrate ($MgSO_4*7H2O$) and zinc sulphate heptahydrate

126 ($ZnSO_4*7H_20$). The hygroscopic compounds were dried in an oven (105ºC) for 4 hours and all
127 compounds were mixed with tap water. When it was necessary, the water was stored in a freezer
128 at 10°C in order to avoid losses of N- ammoniacal by volatilization. However, the water was not
129 stored for more than 3 days. The wastewater was prepared five times during the experiment in
130 order to provide the same initial concentrations of contaminants to all treatments. All data and
131 the standard deviation can be seen in Table 1.

132 **2.1 Cork preparation**

133 High density cork granulates generated during the production of wine stoppers were
134 provided by the Catalan Cork Institute (ICSURO). The granulate extracted from cork oak trees
135 (Q. *suber*) is the commonly used material to seal wine bottles. This material is also called "cork-
136 wood" and it is a mixture of the denser part of the cork, which has part of the back of the cork
137 bark (woody part), part of the belly (part that is in contact with the tree) and the one that is
138 understood by cork. The cork for the test was washed 3 times with demineralized water, dried in
139 an oven for 48 hours at 105°C and sieved to obtain PS of 4 mm and 8 mm.

140 **2.2 Cork characterization**

141 A Fourier Transform Infrared Spectroscopy (FTIR) test was performed using Cary 630
142 FTIR according to the internal protocol PNTM 7.5-54 by triplicates (Ortega-Fernández et al.,
143 2006; Prades et al., 2010; Miranda et al., 2013). Chemical constitution analysis methods have
144 previously been described by Jové et al., 2011. The elemental analysis of C, H, N and O were
145 performed with 4 samples (replicates), using elemental analyser EuroVector EuroEA3000
146 equipped for analysis of CHNS.

147 The determination of point of zero charge for each PS of cork (4 mm and 8 mm) was
148 based on the immersion technique (adapted from Bourikas et al., 2003; Hafshejani et al., 2016;
149 Fiol and Villaescusa, 2009). The pH value at the point of zero charge of cork was determined by
150 adding 250 ml 0.1 M NaCl solution into a series of 500 ml plastic flaks. The initial pH of the
151 aqueous solutions was adjusted in the range of 1-10 by the addition of HCl (0,5-1M) or NaOH

(0,5-1M). After the pH adjustment, 15 grams of cork were added to each flask and the suspension was shaken for 24 hours, at 40 rpm and 22 ± 1◦C. The solution was finally filtered on 0.45 mm cellulose acetate membrane filter and the final pH was measured using digital pH meter (Metrhom) standardized by NBS buffers. The experiment was performed in triplicates. The variation of pH (ΔpH = initial pH – final pH) was plotted versus initial pH.

2.3 Batch studies

The batch studies were carried out at the Department of Bioscience - Aarhus University (Denmark). For all batch experiments, constant conditions for the initial concentration of contaminants (Table 1), adsorbent dosage (100 g L^{-1}) and temperature (20°C ± 1°C) were maintained. The experimental design was based on the following factors and levels: PS: 4 mm and 8 mm, pH: 3,5,7,9 and CT: 0 – 24 hours. All the experiments were performed in triplicates. Control samples, without cork, were used to adjust the results.

2.3.1 Effect of particle size and pH on the release of organic carbon

The initial pH of synthetic hydroponic wastewater (SHW) was adjusted at different pH values (3, 5, 7, 9) by using HCl (0,5-1 M) and NaOH (0,5-1 M) and the SHW was characterized in order to know if initial concentrations were in accordance with the literature range (Table 1). Initial values of organic carbon were considered to be zero. The adsorbent dosage of 100 g L^{-1} was achieved adding the desired amount of adsorbent and aqueous solution into plastic flasks (250 mL). The suspensions were shaken at 40 rpm at 20±1°C during 24 hours. The solutions were filtered and immediately, the final pH of filtered samples was measured using a HACH digital probe and the non-purgeable organic carbon (NPOC) was measured.

A factorial ANOVA (4 x 2) was performed in order to analyse main effects and interaction for significance of 4 mm and 8 mm PS and pH 3, 5, 7 and 9 on the release of organic carbon (OC_I - described in section 2.4.1). Post Hoc test (Tukey HSD 5%) were carried out just for the pH independent variable (more than 2 levels) in order to determine the significance of the differences between the means across the levels.

2.3.2 Kinetics

Kinetic experiments were conducted under pH 7, normally based in hydroponic wastewaters (Table 1), by varying the CT: 0.5, 1, 3, 12, 24 hours following the previously methods described before. After the pre-established CT, the samples were filtered using a cellulose membrane filter, and non-purgeable organic carbon (NPOC) analysis were performed.

A factorial ANOVA (5 x 2) was performed to analyse the effects of PS (4 mm and 8 mm) and CT (0.5, 1, 3, 12 and 24 hours) on the release of organic carbon. Post Hoc test (multiple comparisons – Tukey HSD 5%) were carried out just for the CT (more than 2 levels) to determine the significance of the differences between the means across the levels. In order to determine the specific relationships between both independent variables (PS and CT) across levels, an analysis of simple effects was conducted, using general linear model.

2.3.3 Organic Carbon release indicators

In order to analyse the data from the batch studies, 3 indicators are proposed. The description of the indicators can be seen below.

- **Organic carbon I** $(\boldsymbol{OC_I}) = NPOC_f * V$

OC_I (mg) = mass balance or the mass of organic carbon released. Where, $NPOC_f$ is the final concentration of non-purgeable organic carbon (mg L^{-1}) and V is aqueous volume of the sample (L).

- **Organic carbon II** $(\mathbf{OC_{II}}) = {}^{OC_I}/_{M}$

OC_{II} (mg of organic carbon / g of cork) = The amount of organic carbon released per gram of cork. Where M is the mass of cork in the samples (g) (adapted from Crespo-Alonso et al.; 2013; Hafshejani et al., 2016; Mor et al., 2016; Rajeswari et al., 2016)

- ***Organic Carbon III*** $(\boldsymbol{OC_{III}}) = {}^{OC_I * 100}/_{MC_i}$

OC_{III} (%) = % of OC released related to total OC in the sample. Where MC_i is the initial mass of organic carbon in the sample. MC_i was calculated considering the elemental

analysis performed (66% of the total mass is organic carbon) and the mass of cork in the sample.

2.3.4 Statistics

As mentioned before, for both experimental stages an ANOVA factorial analysis was carried out using the software IBM SPSS Statistics (version 23), in order to understand the main effects of PS, CT and pH on the chemical release of organic carbon. Only the indicator OC_I was used for statistical analysis.

Moreover, the effects on each factor have been individually analysed by a "trial and error" approach. Therefore, for kinetics studies, the design of the needed experiments was carried out using Design Expert® (Design-Experts Software Version 7.0). The DoE technique allows for verifying whether or not there is a synergistic effect between the variables on the final response (Montgomery, 2007; Formosa et al., 2012)., and which parameters can influence the release of organic carbon (OC_I) to a greater extent. The objective was to quantify the results according to the PS and CT which are related with the kinetics. On this manner, a desirable OC_I can be obtained by varying the parameters under study (i.e.: PS and/or CT). The statistic approach was a response surface methodology (RSM), specifically a historical data in order to further perform an optimization process by using the results previously obtained. The analysis of DoE results is based on the analysis of variance (ANOVA) (Montgomery, 2007).

3 RESULTS AND DISCUSSION

3.1 Characterization of cork

3.1.1 FTIR

Cork is mainly composed by suberin and lignin (Miranda et al., 2013). Based on the FTIR spectra (Figure 1), most characteristic absorption bands were between 2800 and 3000 cm-1, corresponding to the link C-H of suberin (Cordeiro et al., 1998), similar to other previous results (Miranda et al., 2013).

The analysed samples showed other bands at 1738, 1630 and 1605 cm-1 corresponding respectively to the C = O bond of suberin and aliphatic acids, C = C bonds of suberin and lignin. Bands from 1600 to 1125 and 1087 to 1035 cm-1 were related, respectively, to different bonds of lignin and C-O bonds of polysaccharides (cellulose + hemicellulose) (Marques et al., 1994). As can be seen in Figure 1, both PS (4 mm and 8 mm) showed similar behaviour regarding their bands and peaks. The heterogeneity of the samples can be explained by some differences between replicates.

3.1.2 Chemical constitution.

The suberin was the major chemical compound, representing 51.3 % of the total composition (Table 2). Together, suberin and lignin represented 65.4 % of the total chemical composition of cork (Q. *suber*) analysed in this present study. The content of lignin and suberin can vary within the same species and for different species (Miranda et al. (2013).

Other authors showed similar results, where the suberin plus lignin of Q. *suber* ranged from 69.8 % - 70.1%. Olivella et al. (2011a and 2011b) results of lignin contents were, respectively, 2.2 and 1.8 times higher than the lignin content of Q. *suber* on our present study. On the other hand, the suberin content of Q. *suber* in our present study (51.3 %) was higher when comparing with the result of Q. *suber* (Olivella et al., 2011a; Olivella et al., 2011b) and 1.35 to 1.8 times higher comparing with Q. *cerris* (Olivella et al., 2011b).

On the other hand, the variation within the same species might be related with the extraction part (belly, back and cork). As can be seen in Table 2, the content of suberin tends to be higher in the belly and cork than in the back part, fact that is in accordance with Jové et al. (2011).

The samples of the present study are a mixture of back layer (woody part), belly (innermost part) and the one that is understood by cork, while the samples of Olivella et al. (2011a and 2011b) were just composed by the belly layer. Therefore, the higher content of

suberin in our study in comparison with other works could be explained by the heterogeneity of our cork sources.

This heterogeneous composition confers cork a unique characteristic and makes it a very interesting natural material to investigate (Olivella et al., 2013a). In fact, not much is known about the influence of the chemical composition of cork on the release of organic carbon.

3.1.3 Elemental analysis

In our samples, carbon was the main element, representing 61.7 % of the total mass of the sample, a similar result obtained from other authors (Olivella et al., 2011b), and can correspond to the ranges of cork and belly (extraction parts) founded in literature (Table 3).

The proportion of material coming from belly and cork layers of our samples might be greater than the back layer, justifying the previously mentioned highest content of suberin. In fact, the content of carbon from Q. *cerris* is slightly lower than the results from Q. *suber*, which might be related to the lower content of suberin mentioned in the previously section.

On the other hand, the organic nitrogen composition might be an issue to be considered in the scope of selecting organic by-products as substrates on NBS for water treatment. The results of Masi et al. (2016) showed an increase of total Kjeldahl nitrogen, which was probably related to the release of organic nitrogen from the substrate (coconut fibber). The organic nitrogen released will be mineralized, and eventually, will change to mineral forms, such as ammonium, nitrates and nitrites. This flow of total nitrogen from the substrate needs to be addressed during the design of such technologies. In our case, the nitrogen content represented less than 1% of the total composition of the samples and was lower than all results founded in the literature (Table 3).

Moreover, the release of greenhouse gases such as CO_2 and N_2O can be increased when organic filter media are used. According to the review made by Maucieri et al. (2017), the increase of organic C and N can lead to higher greenhouse gases emissions in CWs, and denitrification process can increase N_2O emissions (Gentile et al., 2008; (Sarkodie-Addo et al.,

279 2003). Consequently, the high C:N ratio from our substrate could help to enhance denitrification
280 and, at the same time, balance the characteristic higher GHG emissions from low C/N ratio
281 hydroponic wastewaters.

282 **3.1.4 PZC**

283 The PZC can be defined as the pH value in which the surface of the biosorbent has zero
284 charge (or the same number of positive and negative charges). Biosorbent surface net charge
285 plays an important role in the sorption/desorption processes, and to explain protonation /
286 deprotonation behaviour in the aqueous medium. As can be seen in Figure 2, performing the
287 immersion technique, the point of zero charge from granulated cork were between pH 5.5 and
288 5.8, respectively, to PS 8 mm and 4mm.

289 Above pH 6, the surface of the samples is negatively charged, mainly because of the presence
290 of phenolic -OH or carboxylic groups (-COOH). However, the results of Fiol and Villaescusa,
291 (2009) showed a point of zero charge of around pH 3,5 regardless the methodology used, with
292 cork waste from wine industry from Spain.

293 As previously mentioned (section 3.1) the chemical composition of cork might vary according
294 the species and the extracted part and, therefore, different chemical compositions might lead to
295 different behaviour of protonation / deprotonation process that can influence the point of zero
296 charge.

297 **3.2 Effect of PS and pH on the release of organic carbon**

298 The extractives of cork include several organic compounds such as waxes, triterpenes,
299 fatty acids, glycerides, phenols and polyphenols. The pH influences the chemical speciation and
300 the diffusion rate of solutes, the dissociation of sorbent functional groups and the sorbent
301 surface charge (Rahmani et al., 2010; Glestanifar et al., 2016). It is assumed that the PS affects
302 the release of organic carbon, since it is directly related to surface area, although this effect
303 might vary according to the initial wastewater pH.

The results indicated that the null hypothesis can be rejected for PS ($F(1,16) = 293 > 4.49$, $p = 0.05$) and pH ($F(3,16) = 9.61 > 3.24$, $p = 0.05$), indicating the existence of main effects. On the other hand, there was insufficient evidence to reject the null hypothesis of interaction effect ($F(3,16) = 2.66 < 3.24$, $p = 0.05$). Therefore, the main effects of PS and pH are discussed below (Figure 3.).

Regardless the pH, as smaller the PS as higher is the release of organic carbon, due to more available surface area. Regarding the effect of pH, the release was not affected from pH 3 to 7, but a significant effect was obtained from pH 7 to 9 (Tukey HSD, 5%), with higher release at pH=9, perhaps due to the deprotonation of phenolic –(OH) or carboxylic groups (–COOH) at pH 8-9. No significant interaction effect was obtained ($p=0.083$). The main effect of PS (95% of the variance) is stronger than the main effect of pH (64% of the variance).

Moreover, 95% of the variance on the release of organic carbon can be attributed to the PS, while 64 % was explained by the variance of initial pH, fact which, suggest that the main effect of PS is stronger than pH. Therefore, comparing each PS across levels of pH, separately, the pH did not affect the release of organic carbon for PS 8 mm. On the other hand, for PS 4 mm, the differences between pH 3-7 and 7-9 were statistically different, decreasing and increasing, respectively. These results might indicate that as lower the PS as greater can be the effect of pH on the release of organic carbon.

3.3 Kinetics

The null hypothesis can be rejected for PS ($F(1,20) = 931.33 > 4.35$, $p = 0.05$) and CT ($F(4,20) = 232.93 > 2.87$, $p = 0.05$), indicating the existence of main effects of CT and PS on the release of organic carbon. The results showed significant effect of PS and CT on the release of organic carbon, increasing with smaller PS ($p<0.05$) regardless the CT. Indeed, the mass of carbon released by PS 4 mm was two times higher than the release of organic carbon by PS 8 mm for all CT, except for CT 3 hours which was 1.7 time higher. This may indicate the

329 presence of a possible inverse exponential relationship between PS and release of organic
330 carbon.

331 The post hoc tests (Tukey HSD, 5%) indicate that the multiple comparisons across levels
332 of CT were significant, increasing the release of organic carbon when the CT increases,
333 regardless the PS (Figure 4).

334 An interaction effect was noticed on the release of organic carbon (PS*CT - $F(4,20) =$
335 $28.87 > 2.87$, $p = 0.05$). All eta squared ($\eta 2$) were greater than 0.14, indicating that both, main
336 and the interaction effects, are representing great influence on the release of organic carbon.
337 However, while the main effects of PS and CT represents 98% of the release of organic carbon,
338 the interaction effect between then represents 85%, suggesting that the main effects of PS and
339 CT are slightly greater than the interaction effect.

340 The pairwise comparison results showed no significant differences on the release of
341 organic carbon in the periods, 3-12 hours and 12-24 hours, for PS 8 mm. However, the release
342 of organic carbon after 24 hours was significantly higher than after 3 hours. Moreover, there
343 was significant differences between all the means across CT, for PS 4 mm. These results
344 indicate that in the period of 3-12 hours and 12-24 hours the release of organic carbon was
345 influenced by PS and/or that there is an interaction between the independent variables. This
346 result might indicate that the CT may have a stronger effect on PS 4 mm than on PS 8 mm, in
347 other words, with smaller PS the effect of CT is higher on the release of organic carbon.

348 Considering the mass of carbon released after 24 hours as the total released it is possible
349 to conclude that approximately 32 % and 11 % of total carbon released took place during the 3
350 to 24 hours period, respectively for PS 4 mm and PS 8 mm.

351 On the other hand, more than 70% of the released organic carbon occurred during the first
352 3 hours, for both PS. Therefore, the release of organic carbon might get slower when the CT
353 increases. In addition, this effect might be stronger with the increase of PS. Considering that
354 the specific surface area and PS are inversely related, the surface area might have an effect not

355 just on the amount of carbon released but also at which the speed that the release of carbon
356 takes place.

357 Table 4 summarizes the results following the RSM obtained using the software Design
358 Expert® for the best fitted model. Both factors (PS and CT) present significant effect on the
359 response (OC_I) in the range of the study. In this case, the best fitted model is a response surface
360 reduced cubic model which presents interaction between the factors under study (PS and CT)
361 with a p-value<0.05. In addition, there is a cubic interaction (see factor CT^2PS and p-vales<0.05
362 in Table 4). Besides, a quadratic and a cubic effect on the response is presented for the CT
363 factor in the range of the study.

364 PS factor does not fit in the proper manner when is in quadratic and/or cubic function, for
365 that reason these terms where discarded on the final equation. In addition, it should have been
366 emphasized that the lack of fit is not significant. Consequently, there is only a 0.01% chance
367 that the model occurs due to noise.

368 All the results derived from the modification of any of the controllable variables can be
369 translated into a predictive mathematical model. This model can quantitatively predict the
370 response within the operating range of controllable variables. It can also give some suitable
371 formulations when a certain response is required. The model only incorporates the statistically
372 significant factors and interactions. Therefore, the mathematical model can be written by the
373 following equation:

374 $$OC_1(mg\cdot) = 5.471 + 2.137 \times CT - 0.506 \times PS - 0.069 \times CT \times PS - 0.163 \times CT^2 + 0.002 \times CT^2 \times PS + 0.004 \times CT^3$$

375

376 Figure 5 presents the surface plot obtained for OCI. An increase of PS or CT lead to a
377 decrease of OC_I. When both factors are increased their combined effect is found to be lower
378 than the expected form considering the sum of each factor separately. Therefore, it can be
379 concluded that there is significant negative interaction between both factors: as higher the PS
380 the lower the response of OC_I, as we previously explained.

3.4 Cork as an organic carbon source for denitrification.

In order to estimate the denitrification provided by granulated cork through the chemical release of carbon, a series of assumption were made, considering the following theoretical stoichiometry for denitrification: 1 g org-C per g nitrate-N (Zhai et al., 2013). Moreover, the density of cork was considered to be 123 Kg /m^3 and 125 Kg /m^3 for PS 8 mm and 4 mm, respectively (Source: internal data from ICSURO). The averages of OCII (mg of organic carbon released / g of cork) at 24 hours were used for the calculations. Indeed, the hydroponic wastewater to be treated (Table 1) and temperatures were considered as the same as the one used at lab conditions.

Therefore, the chemical release of organic carbon, considering a batch bio filter with unknown dimensions filled up with 1 m^3 of cork, would be approximately 265 g (PS 4mm) and 120 g (PS 8 mm) after 24 hours. If one considers that all the organic carbon released is consumed by the denitrification process it means that 3.9 m^3 and 1.8 m^3 of hydroponic wastewater could be treated, using respectively PS 4 mm and 8 mm.

It is well known that PS is a crucial parameter when NBS are designed for wastewater treatment. The PS besides influencing the hydraulics of the system, also strongly affect the performance of contaminants removal by adsorption, complexation and precipitation and as well by microbiological process since influence the biofilm growth (Vymazal, 2007[b]; Wu et al., 2015). The results mentioned above showed that by using a 4 mm PS, the amount of water treated after 24 hours of batch treatment was more than 2 times that for PS 8 mm. Moreover, results of Capodici et al. (2014) showed that the PS might have a greater influence on organic carbon release than the total organic carbon content itself. The author compared several materials, including cork. Cork presented the lowest result regarding the release of organic carbon, even though it had the highest total organic carbon content. Fact, which were related to its biggest PS of cork in comparison with the other materials. Moreover, the kinetics results highlighted that organic carbon release from granulated cork decreases with time, and this effect is stronger when the PS increases. Therefore, the effect of PS on the release of organic carbon

should be considered when NBS treatments are designed using cork as filter media and organic carbon source.

As can be seem in Figure 6., the chemical release of organic carbon was less than 1% of the total content of carbon after 24 hours, for both PS, suggesting that cork could be suitable for a long-term carbon organic source.

According with previously results, the release of organic carbon gets slower with time, fact which could be a limitation regarding cork long-term efficiency as carbon source. In the present study, after 24 hours 2.12 (for PS 4 mm) and 0.98 (for PS 8 mm) mg of organic carbon/g of cork was released. In the other hand Capodici et al. (2014) results showed that, after 50 hours the peak of carbon release was reached being 5.6 mg of organic carbon / g of cork. After 50 hours the increment of carbon released was slower and linear. Those results suggest that even that the organic carbon release gets slower with time it keep taking place, fact which highlight the importance of models to predict it.

Moreover, it is important to take into account that the performance of cork as carbon source will be influenced also by real scale features such as, type of treatment (bio filters, CWs, green walls and others), design and operation factors (type of flow, saturated or unsaturated conditions, retention time, hydraulic and contaminants load among others), cork features (chemical composition) and environmental conditions (temperatures). Therefore, further studies on long-term efficiency of organic carbon release from cork are needed.

4 CONCLUSIONS

The main compound and element of cork are Suberin and carbon, representing respectively, more than 50% and 60% of the samples composition. Also, when comparing the results with other researches, it was noticed that might be a variance on the content of suberin across species, within the same species and depending on the extraction part (Belly, cork and back). Furthermore, the lignin content seems to vary within Q. *sube*r specie. However, no statistical analysis was performed to validate this hypothesis. Nevertheless, as not much is

known about the influence of the chemical composition of cork on the release of organic carbon, further researches on it might facilitate standards to ensure an efficient performance of cork as carbon source in accordance with its chemical constitution.

The point of zero charge of cork was between pH 5 and 6, which was different than the result founded in literature (3.5). This difference was attributed to the variance of cork chemical composition which might lead to different behaviour of protonation / deprotonation.

As smaller the PS as higher is the organic carbon released, regardless the pH or CT. Regarding the pH main effect, the results suggest that as lower the PS as stronger the effect of pH on the release of organic carbon. The kinetics results showed that as the CT increases the release of organic carbon is increased as well, regardless the PS. However, an interaction effect between PS and CT was noticed, indicating that as smaller the PS becomes the higher the effect of CT has on the release of organic carbon. In addition, more than 70 % of the carbon released took place during the first 3 hours for both PS, indicating that the release of organic carbon might get slower as CT increases. Those results highlight that the effect of surface area affects the amount carbon released and as well the velocity that the carbon is released.

When using cork as carbon source of NBS treating wastewater, the effect of PS on the release of organic carbon can play an important role at designing such systems. Estimations showed that the amount of water treated by using PS 4mm was more than 2 times that would be for PS 8 mm, considering that all carbon released would be consumed by denitrification. In this regard, the results of surface response methodology indicate that optimization could be performed to facilitate the design of technologies considering the interaction between PS and CT at releasing organic carbon.

Using cork as a source of carbon for denitrification seems to be a promising alternative to reduce costs and environmental hazard of NBS treating wastewaters with low carbon content and high nitrates. By using organic substrates, the development of microbiota also might be facilitated and thus, microbiological removal process. However, this practice might lead to losses of hydraulic conductivity and adsorption surface area, fact that can influence treatment

efficiency. Moreover, long-term conditions can influence the cork behaviour at releasing organic carbon, since other external factor will be involved (type of treatment, design and operation factors, cork features and environmental conditions). Therefore, validating the use of cork as a carbon source for denitrification at real and long-term scales can be an interesting line of research. Furthermore, the effect of such practice on the release of greenhouse gases also should be considered.

Nerveless, the reuse of organic by-products as filter media seems to be an environmental and economic friendly alternative to enhance denitrification in NBS. This approach can help preserve natural capital, reduce the dependency of external inputs, treatment costs, increase self-efficiency, all of it leading to a sustainable technological development in the scope of wastewater treatments.

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Table 1. Composition of hydroponic synthetic wastewater (Adapted from Prystay and Lo, 2001; Koide and Satta, 2004; Huett et al., 2005; Taylor et al., 2006; Park et al., 2009; Gagnon et al., 2010; Gruyer et al., 2013; Dunets et al., 2015; Park et al., 2015; Chang et al., 2016)

		Synthetic Hydroponic wastewater	**Literature RANGE**	
Compounds	**Unit**	Average ([a]SD ±)	Min	Max
N total	*mg* L^{-1}	70.89 ± 1.1	2.8	122.0
NO_3^--*N*	*mg* L^{-1}	66.28 ± 1.0	10.0	414.0
NH_4^+-*N*	*mg* L^{-1}	4.61 ± 1.4	0.8	36.7
PO_4^{-3}-*P*	*mg* L^{-1}	11.01 ± 3.3	0.7	99.3
[b]K^+	*mg* L^{-1}	189.18	13.0	459.0
[b]Na^+	*mg* L^{-1}	83.00	83.0	108.0
[b]Ca^{2+}	*mg* L^{-1}	123.52	21.0	295.0
[b]Mg^{2+}	*mg* L^{-1}	90.00	10.0	105.0
[b]Cl^-	*mg* L^{-1}	41.00	3.9	80.0
[b]Zn^{2+}	*mg* L^{-1}	0.50	0.03	1.4
pH		9.6 ± 0.08	5.5	7.3
[c]*EC*	dS m^{-1}	2.2 ± 0.05	1.3	2.3
[d]SAR	meq L^{-1}	1.96	1.8	2.0

[a] Statistical deviation (SD) was performed using IBM SPSS. [b] Equal to concentration calculated by the amount of compound used (same for all water prepared). [c]Electrical conductivity. [d] SAR: The Sodium Adsorption Rate was calculated based on (Pescod,1992). To determine the literature range, the SAR was calculated considering the values of Na^+, Ca^{2+} and Mg^{2+} found in the following papers: Koide and Satta, 2004; Park et al., 2009.

Table 2. Chemical composition of cork granulate.
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Table 2. Comparison of chemical composition from granulated cork of present study with literature results.

	Species		*Extraction part		
Chemical compounds (%)	Q. *Cerris*	Q. *Suber*	Cork	Belly	Back
Suberin	[2]28.5	[1]51.3 (± 0.2) [2]44.1 [3]38.5	[4]34.4 – 48.7 [5]33.5 – 48-7	[4]33.5 – 53.1	[4]21.1 – 40.7 [5]21.1-40.7
Total lignin	[2]28.1	[1]14.1 (± 0.6) [2]25.7 [3]31.6	[4]14.6 - 25.3 [5]13.4 - 31	[4]14.9-31	[4]18.9 – 28 [5]23.9 – 27.9
Suberin + Lignin	[2]56.6	[1]65.4 [2]69.8 [3]70.1	[4]54.4-71 [5]54.7 – 71.4	[4]55 – 69.8	[4]41.6 – 64 [5]49 – 64.6

* Range: lower and the highest results from different origin area for each extraction part. [1]Results of the present study. The Standard deviation was calculated with triplicates using the software IBM SPSS (values in brackets). [2]Adapted from Olivella et al., 2011b. [3]Adapted from Olivella et al., 2011a. [4]Adapted from Jové et al., 2011. [5]Adapted from Olivella et al., 2013a.

Table 3. Elemental composition of cork granulate.
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Table 3. Comparison of elemental composition of granulated cork found on our study and literature results.

	Species		*Extraction part		
Elements (%)	***Q. Cerris***	***Q. Suber***	***Cork***	***Belly***	***Back***
Carbon (C)	[2]50.7	[1]61.7 (± 0.97) [2]61	[3]58.5 – 63.1	[3]60.2 – 62.5	[3]51.5 – 59.7
Hydrogen (H)	[2]7.3	[1]7.7 (± 0.2) [2]8.7	[3]7.1 - 8	[3]6.8 - 9	[3]6.6 – 7.3
Nitrogen (N)	[2]1.73	[1]0.68 (± 0.05) [2]1.7	[3]1.3 – 2.1	[3]1.3 – 3.1	[3]1.2 - 2
Oxygen (O)	[2]31.4	[1]29.8 (± 1.14) [2]22.57	[3]26.8 – 36.1	[3]28.4 – 36.1	[3]31-42

[*] The range: lower and the highest results from different origin area for each extraction part.[1]Results of the cork granulated used for this study (Q. *Suber*). The Standard deviation was calculated with triplicates using the software IBM SPSS (values in brackets). [2]Adapted from (Olivella et al., 2011)[3] Adapted from (Olivella et al., 2013).

Table 4. NOVA - Response Surface Reduced Cubic Model.
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Table 4. ANOVA for Response Surface Reduced Cubic Model.

Factors	**Sum of squares**	**[a]df**	**Mean square**	**F-value**	**[b]cv**	**[c]ρ-value**
Model	225.73	6	37.62	329.91	2.60	$\rho < 0.05$
PS	5.10	1	5.10	44.72	4.35	$\rho < 0.05$
CT	41.45	1	41.45	363.47	4.35	$\rho < 0.05$
PS*CT	11.57	1	11.57	101.47	4.35	$\rho < 0.05$
CT^2	8.95	1	8.95	78.49	4.35	$\rho < 0.05$
CT^2PS	0.99	1	0.99	8.66	4.35	$\rho < 0.05$
CT^3	12.45	1	12.45	109.23	4.35	$\rho < 0.05$
Lack of Fit	0.33	3	0.11	0.99	3.10	$\rho > 0.05$
Pure Error	2.29	20	0.11	-	-	-
Total	228.36	29	-	-	-	-

[a]df = Degrees of freedon. [b] Critical value of F distribution. [c]$\rho < 0.05$ = significant. $\rho > 0.05$ = not significant

Figure 1. FTIR results - cork granulate Quercus suber

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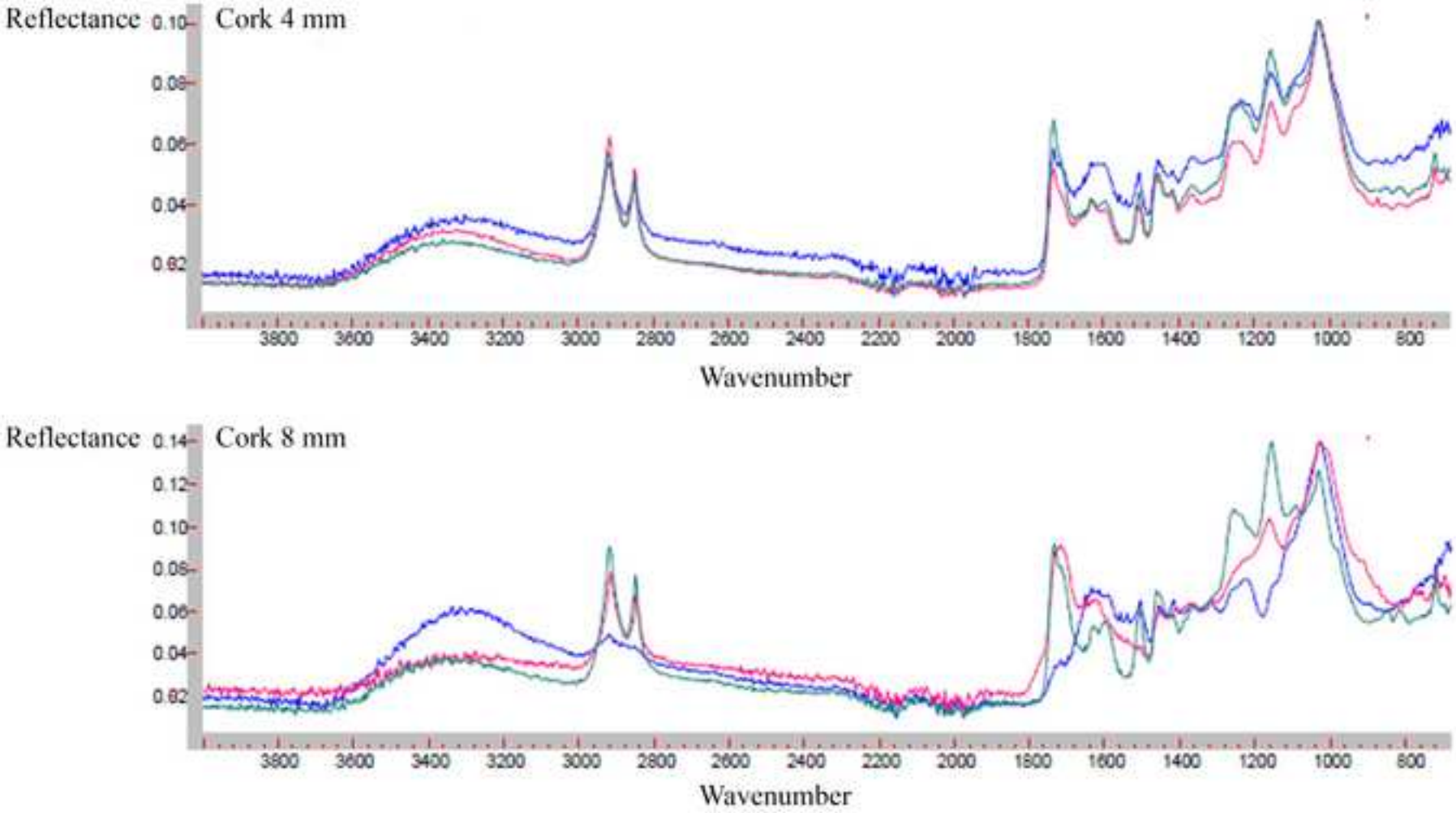

Figure 2. PZC - Immersion Technique.
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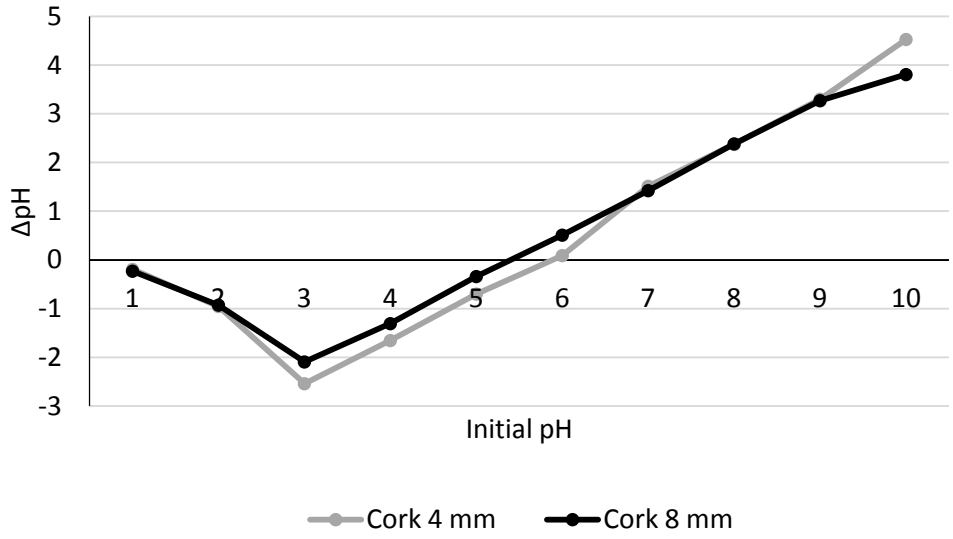

Figure 3. Effect of PS and pH on the release of organic carbon.
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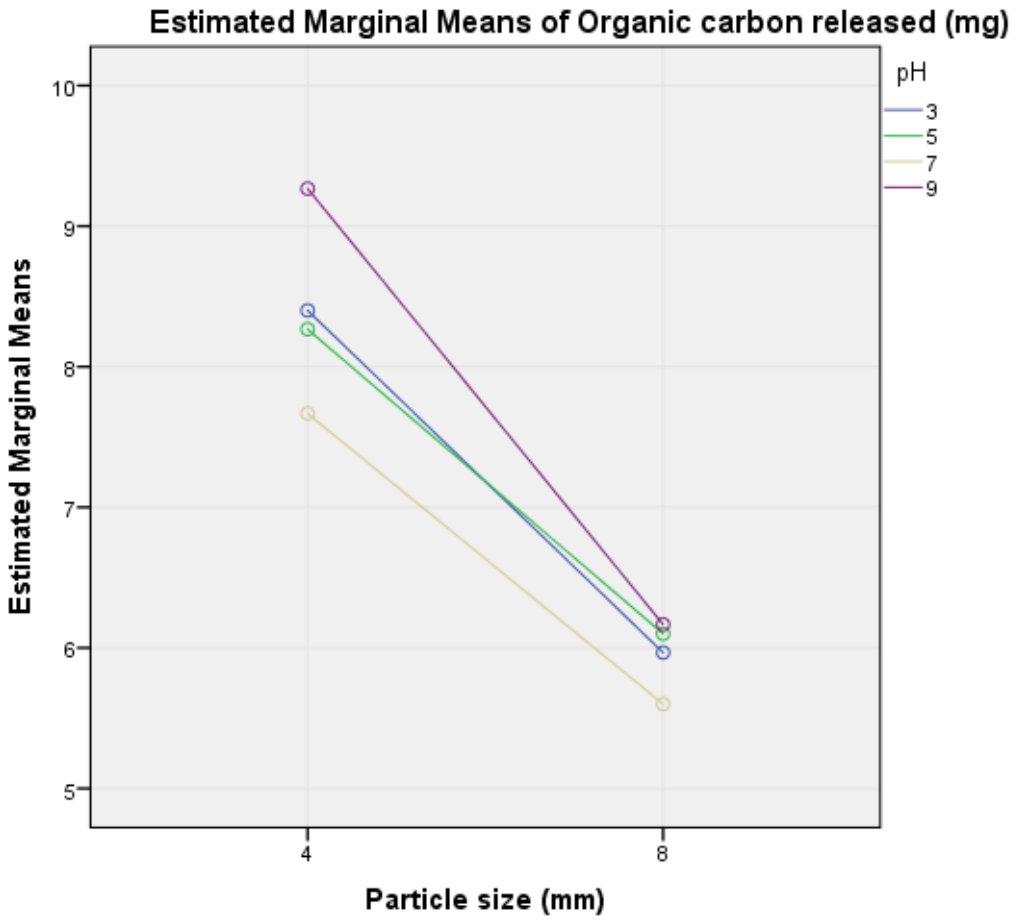

Figure 4. Main effect of CT on the release of organic carbon.
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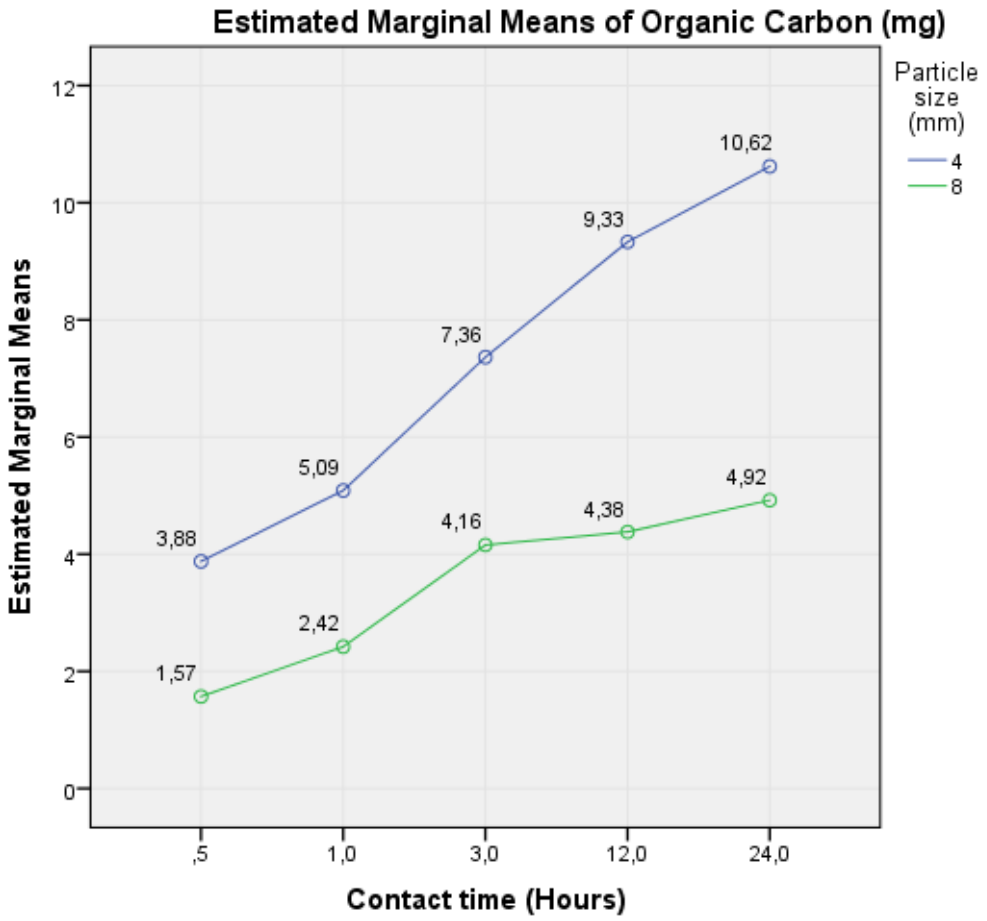

Figure 5. Surface plot of the OCI as a function of CT and PS.
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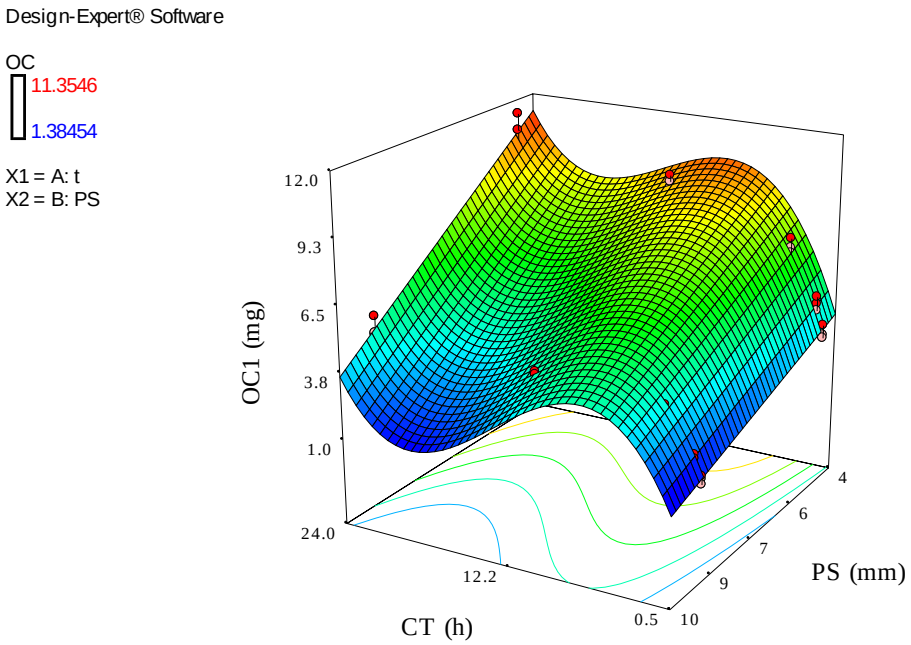

Figure 6. Effect of CT on OCIII (described in section 2.4.1).
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